Annals
of the
Missouri Botanical Garden

Vol. 8 SEPTEMBER, 1921 No. 3

STUDIES IN THE PHYSIOLOGY OF THE FUNGI

XIV. Sulphur Nutrition: The Use of Thiosulphate as Influenced by Hydrogen-Ion Concentration[1]

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Introduction and Historical Review

Sulphur is an essential element in the cell because it is a component part of certain indispensable proteins. The metabolism or decomposition of various sulphur compounds by bacteria has been investigated by several workers, but the metabolism of the fungi with reference to such compounds has been little studied and is poorly understood. The activity of the sulphofying organisms of the soil has been a field for considerable investigation in recent years. It has been shown that these organisms bring about the change of the organic sulphur of the soil into sulphates which become available for crops. The oxidation of sulphur by such organisms has been shown to be of value in making available the phosphorus of mineral phosphates (McLean, '18).

Sulphur metabolism may also play a beneficial role in sewage disposal in that certain species of bacteria may cause the oxidation of sulphur and of hydrogen sulphide to sulphates, thus reducing the amount of odor. Attempts have been made to apply

[1] An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University, and submitted as a thesis in partial fulfilment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

the production of hydrogen sulphide to water analysis, the assumption having been that the hydrogen sulphide produced is proportional to the pollution. The literature pertaining to such attempts will be found in the paper by Myers ('20). The fate of hydrogen sulphide produced in putrefactions and other processes under the influence of "sulphur bacteria" has also been investigated by several workers, chiefly Winogradsky ('87, '88), Keil ('12), Düggeli ('19), and Skene ('14). In addition to the hydrogen sulphide produced in putrefactions, another source of this compound is from the reduction of sulphates by microorganisms such as bacteria and yeasts. The literature pertaining to sulphate reduction by bacteria and yeasts is discussed in papers by Lederer ('13) and Tanner ('17, '18).

An interesting phenomenon resulting from the sulphur metabolism of many organisms is the deposition of sulphur in the cells. This has been shown for bacteria by Cramer ('70), Cohn ('75), Winogradsky ('88), Keil ('12), Miyoshi ('97), Hinze ('03), and Lidforss ('12). Wille ('02) has denied the occurrence of sulphur in *Thiothrix*. Jonsson ('89) was the first to describe sulphur in the hyphae of fungi. He describes refractive bodies which are not sulphur, but oily bodies containing sulphur, in the hyphae of *Penicillium glaucum* growing on N/10 H_2SO_4 solution. Raciborski ('06) and Kossowicz and Loew ('12) have also described the presence of sulphur in the hyphae of fungi.

That sulphocyanate compounds are available for the growth of some bacteria and fungi seems established from the work of Beijerinck ('04), Munro ('86), Czapek ('03), Puriewitsch ('12), Kossowicz and Gröller ('12), Fernbach ('02), and Sauton ('10), though Holschewnikoff ('89) did not observe a decomposition of such compounds by bacteria, and Nägeli ('82) states that ammonium sulphocyanate can not be assimilated by fungi.

The relation of many organisms to the thiosulphates has been investigated by a considerable number of workers. Hydrogen sulphide is most generally produced as a result of the action of the organisms on these compounds as was found by Holschewnikoff ('89), Beijerinck ('00, '04), Petri and Maassen ('93), Saltet ('00), Nathansohn ('02), Sasaki and Otsuka ('12), Lederer ('13), and Tanner ('17) in the case of bacteria; Neuberg and Welde ('15), Beijerinck ('95), Tanner ('18), and Stange ('15) in the case of yeasts; and Raciborski ('06) and Kossowicz and Loew ('12)

in the case of the fungi. Gehring ('15), Lockett ('14), and Lieske ('12), in their investigations with certain bacteria, report the use of the thiosulphate but not a production of hydrogen sulphide. Gehring found the gases produced to be 5.5 per cent of carbon dioxide and 94.5 per cent of nitrogen, while Lieske found 20 per cent carbon dioxide and 80 per cent nitrogen. Bokorny ('12) and Nägeli ('82), working respectively with yeasts and fungi, report the growth of the organisms on sodium thiosulphate. Buchner and Hahn ('03) have found that the juice from pressed yeast has a reducing action on sulphur and thiosulphate.

With filamentous fungi on the varying solutions containing thiosulphate employed by Raciborski and Kossowicz and Loew, other products than hydrogen sulphide as a result of the growth of the organisms were sulphates, sulphites, extracellular sulphur, intracellular sulphur, and polythionates. All of these substances, except the sulphite, for which no tests were made, were found in one or the other of my cultures. The crystallization of the sulphur in the old hyphae of *Aspergillus niger* in the form of double pyramids as reported by Raciborski, has also occurred noticeably in some of my cultures in which the concentration of the thiosulphate was 2 per cent or more.

It is only in very recent times that work has been done dealing with the actual hydrogen-ion concentration of media and the shifting of the hydrogen-ion concentration due to metabolism. Clark and Lubs ('17) have given the final reaction of a culture of *Aspergillus niger* as P_H 1.7. Currie ('17) has given the critical hydrogen-ion concentration for *Aspergillus niger* on a solution very similar to that employed in experiments 16 to 21 of this paper as P_H 1.4 to 1.6. Steinberg ('19) reported the final hydrogen-ion concentration for the same fungus on Pfeffer's solution as P_H 1.0–2.0 in most cases. On a modified Pfeffer's solution, the highest hydrogen-ion concentration of any culture in my experiments was P_H 1.5, though the final reaction was greatly influenced by the initial P_H of the medium. Meacham ('18) stated that the limiting acidity appears in the region of P_H 1.7 for 4 wood-destroying fungi with which he worked. Zeller, Schmitz, and Duggar ('19) gave the changes in hydrogen-ion concentration of several liquid media due to the growth of a number of wood-destroying fungi. The general tendency was

to increase the active acidity during growth, though there were exceptions. They call attention to the fallacy of combining the results obtained from a few organisms and drawing general conclusions as to the relation of hydrogen-ion concentration and growth of a group of fungi. Duggar, Severy, and Schmitz ('17) stated that *Aspergillus niger* shifts the reaction of certain plant decoctions to a hydrogen-ion concentration of about 10^{-3}.

Gillespie ('18) has shown the limiting acidity for *Actinomyces scabies* (*chromogenus*) to be between P_H 4.8 and 5.2. Growth was accompanied by a marked decrease in the acidity.

Ayers and Rupp ('18), in an explanation of reversions of reaction of culture media by organisms of the colon-aerogenes group, ascribed the simultaneous production of acid and alkali in an inorganic medium to the production of organic acids from the sugar with the subsequent formation of alkaline carbonates or bicarbonates from the organic acids. Waksman and Joffe ('20) are of the opinion that Actinomycetes are not able to produce any appreciable quantities of acid from the carbohydrates which they employed, but that the change in reaction of the medium is due to the source of nitrogen. Their explanation of the alkalinity produced in a nitrate medium is that in the reduction of the nitrate to nitrite, the oxygen split off is united with the hydrogen or other reducing substances of the medium, thus tending to reduce the hydrogen tension of the medium. Boas and Leberle ('18, '18a, '19, '20), in a series of articles on the production of acid by molds and yeasts, have found that both the carbon and nitrogen sources may influence the hydrogen-ion concentration resulting from metabolism, that with the same carbon source and different sources of nitrogen, for example, the greatest hydrogen-ion concentration of the solution on which *Aspergillus fumigatus* has grown may vary between P_H 1.56 and 5.79.

Methods

The methods employed in the part of this work which was completed in 1915–17 (see page 242) and in that completed in 1919–20 are essentially the same. The cultures during the first part of the work were grown in 100-cc. Jena flasks, using water doubly distilled from glass, which usually gave a conductivity test from 1.0 to 1.3 $\times 10^{-6}$, with .8 $\times 10^{-6}$ the best obtained at any time. The water used during the latter part of the experiments was doubly distilled from glass and most of it was

about P_H 5.2. The chemicals used were Merck's Blue Label Reagents in all cases except the mono- and dibasic potassium phosphates in the experiments of 1915–17. The monobasic potassium phosphate was used without recrystallization in the first experiments, but in the later experiments a salt recrystallized several times that gave the Sörensen coefficient of P_H 4.529 for a 1/15 molecular solution was employed in all the culture solutions.

The Jena flasks and the 120-cc. Non-Sol flasks of the later series of experiments were carefully cleaned in an acid dichromate solution and thoroughly rinsed in distilled and redistilled water.

Inoculations were made by transferring spores from a potato agar slant to 10 cc. of sterile distilled water until a heavy spore suspension was obtained. In the first experiments .2 cc. of this suspension was added under sterile conditions to each flask, while in the later experiments, .5 cc. of the inoculum was used.

All controls were uninoculated, sterile solutions which were kept under the same conditions as the experimental flasks. The salt and carbohydrate components of each solution are given on the basis of a 50-cc. culture. In all cases sufficient water was added to make the volume 50 cc. The production of H_2S was determined by suspending in the neck of each flask a strip of filter-paper which had been soaked in lead acetate. These strips were sterilized by soaking in a nearly saturated simmering lead acetate solution and then transferred to sterile dishes.

The determination of sulphates in solution was made by adding $BaCl_2$ after the solution had been acidified with dilute HCl to prevent the precipitation of phosphates.

The titrations of the thiosulphate in solution were made with N/10 or N/100 iodine. This standard titration method furnishes a definite quantitative method for the determination of the thiosulphate decomposed by each organism. The starch and iodine solutions were prepared and standardized according to directions given in the 'Analytical Chemistry' of Treadwell and Hall. Ten cc. of the solution from each culture, to which was added 1 cc. of starch paste, were used in the titrations. Dry weights were obtained by drying to a constant weight in an oven at 105° C.

To follow the successive changes in hydrogen-ion concentration,

thiosulphate content, growth, and the use of the sugar, a sufficient number of flasks were inoculated so that 3 flasks could be removed at stated intervals and the several determinations made from these cultures. It is impossible to make all the determinations throughout from the same flasks, which would be a preferable method if convenient, though the results obtained indicate that the method employed is satisfactory.

Hydrogen-ion concentrations were determined colorimetrically, using the standard solutions as recommended by Clark and Lubs ('17). As the color produced in the solution necessitated the use of a colorimeter, a DuBoscq micro-colorimeter was employed of the type and according to the method described by Duggar ('19).

EXPERIMENTAL RESULTS

A. AVAILABILITY OF SOME COMPOUNDS AS SOURCES OF SULPHUR

The experimental work presented in this paper was in progress several years, experiments 1–15 having been completed during the period 1915–1917 at the University of Wisconsin and the remainder of the experiments at the Missouri Botanical Garden during the sessions 1919–20 and 1920–21. Several of the solutions of the first 15 experiments are the same as those used by Kossowicz and Loew and Kossowicz and Gröller. Only 15 of the 32 experiments performed during the first period of the work are presented, the results obtained being in more or less general agreement throughout. The cultures were placed in a large constant temperature dark room with a variation of from 22 to 25° C. The fungi have been designated in the tables by abbreviations as, *Aspergillus niger*, A. nig.; *Penicillium glaucum*, P. gl.; *Penicillium cyclopium*, P. cycl.; and *Botrytis cinerea*, B. cin.

The extent of the darkening of the lead acetate paper is taken as the criterion of H_2S production which is expressed roughly by the figures 0, 1, 2, 3, 4. An attempt is made to give a general indication of the relative spore production by the use of the same figures. The tabulated data as to the presence of sulphates is given by the plus and minus signs without any attempt to express the relative production of such compounds. This system of notation appears in all the tables.

The nutrient solution used to obtain the results given in table

1 contained the following amounts of the salts per 50-cc. culture: $Na_2S_2O_3$ M/10, 8.1 cc.; KH_2PO_4 M/6, 1.1 cc.; KNO_3 M/4, 3.9 cc.; NH_4Cl M/4, 3.7 cc.; $MgCl_2$ M/60, 2.9 cc.; $FeCl_3$ M/1000, .5 cc.; dextrose M/1, 2.8 cc. as the source of carbon in experiment 1; sucrose M/1, 2.8 cc. as the source of carbon in experiment 2. It will be observed from table 1 that where equi-molecular volumes of dextrose and sucrose are employed differences in

TABLE I

GROWTH AND RELATIONS OF CERTAIN FUNGI ON MEDIA CONTAINING SODIUM THIOSULPHATE. TIME INTERVAL OF CULTURES, 7 WEEKS

	Experiment 1 .2 per cent $Na_2S_2O_3$ and dextrose				Experiment 2 .2 per cent $Na_2S_2O_3$ and sucrose			
Fungus	A. nig.	P. gl.	B. cin.	Check	A. nig.	P. gl.	B. cin.	Check
No. cultures	2	2	2	1	3	4	2	1
Dry wt. (gms.)	.0941	.1351	.1117		.1722	.1800	.2077	
Cc. N/100 I	5.2	13.9	12.8	15.6	1.0	13.5	11.1	15.8
% $Na_2S_2O_3$ decomposed	66.6	10.9	18.0		93.6	14.5	29.7	
H_2S	2	3	4		2	3	2	
Sulphates	+	+	+	—	+	+	+	—
Sporulation	3	4	4		3	4	3	
Cc. N/10 KOH	.9			.3	1.3			.3
Cc. N/10 H_2SO_4		.1	.1			.1	.1	

growth have occurred which may be due to sucrose as a better source of carbon, or to the greater total quantity of carbon supplied. These organisms are able to use the thiosulphate with the production of very noticeable quantities of H_2S and sulphates. Using phenolphthalein as an indicator, a production of acidity is indicated for *Aspergillus*, while *Penicillium* and *Botrytis* produce an alkalinity of the solution.

Two different nutrient solutions as employed by Kossowicz and Loew were used in experiments 3 and 4. All salt concentrations are given for 50-cc. cultures. The solution for experiment 3 was as follows: $Na_2S_2O_3$ M/1, 1.2 cc.; KH_2PO_4 M/60, 2.2 cc.; $(NH_4)_2HPO_4$ M/6, 2.2 cc.; KNO_3 M/4, 4.5 cc.; NH_4NO_3 M/1, 1.2 cc.; $MgCl_2$ M/60, 2.0 cc.; $CaCO_3$ M/100, 1.0 cc.; $FeCl_3$ M/1000, 1.0 cc.; dextrose M/1, 6.9 cc.

The solution for experiment 4 was as follows: $Na_2S_2O_3$ M/1,

4.0 cc.; $(NH_4)_2HPO_4$ M/6, 27.7 cc.; KH_2PO_4 M/6, 2.2 cc.; $MgCl_2$ M/1, 1.0 cc.; $CaCO_3$ M/100, .5 cc.; $FeCl_3$ M/1000, .5 cc.; sucrose M/1, 7.3 cc. The length of the experiment was 4 weeks for number 3 and 5 weeks for number 4.

TABLE II

GROWTH AND RELATIONS OF CERTAIN FUNGI ON MEDIA CONTAINING SODIUM THIOSULPHATE

	Experiment 3 .6 per cent $Na_2S_2O_3$				Experiment 4 2 per cent $Na_2S_2O_3$			
Fungus	A. nig.	P. gl.	B. cin.	Check	A. nig.	P. gl.	B. cin.	Check
No. cultures	5	5	6	1	4	4		1
Dry wt. (gms.)	.0448	.4046	.2509		.4152	.6164	none	
Cc. N/10 I.	0	1.9	.9	3.0	2.1	3.8		8.0
% $Na_2S_2O_3$ decomposed	100	36.6	70.0		73.7	52.5		
H_2S	—	—	—	—	0	0		0
Sulphates	+	+	+		+	+		—
Sporulation					1	3		

On the solution employed in experiment 3 *Aspergillus* made very little growth, yet all the thiosulphate had disappeared from the solution. The sulphates determined as $BaSO_4$ were so low that other tests were tried to discover the changes which had proceeded, and these seem to indicate the production of a tetrathionate. The tests were made as follows: To the solution in which there was no thiosulphate, $BaCl_2$ was added and the sulphate formed was caught in a Gooch crucible. The filtrate was oxidized with bromine and $BaCl_2$ again added. A second white precipitate was formed which indicates the production of a polythionate. Qualitative tests with mercurous nitrate gave a yellow precipitate which might be produced by either the tetra- or pentathionate. Potassium hydroxide added to the solution gave no precipitate of sulphur which should be the case if the pentathionate were present. Hence, it appears that the tetrathionate was in the solution.

In the 2 per cent concentration of thiosulphate in the solution as employed in experiment 4, the toxic effects of the thiosulphate

are evident for *Botrytis*. This organism made fairly good growth on the .6 per cent solution of the previous experiment. Since no H_2S was produced on the 2 per cent thiosulphate solution, an attempt was made to account for all the sulphur by titrating for the thiosulphate, precipitating the sulphate in solution as $BaSO_4$,

TABLE III

GROWTH AND RELATIONS OF CERTAIN FUNGI ON MEDIA CONTAINING SODIUM THIOSULPHATE. TIME INTERVAL OF CULTURES, 4 WEEKS

Fungus	No. of cultures	Dry wt. (gms.)	Cc. N/10 iodine	Per cent $Na_2S_2O_3$ decomposed	H_2S	Sulphate	Sporulation	Cc. KOH N/10
Experiment 5 —5 per cent $Na_2S_2O_3$								
A. nig.	4	.3400	7.6	67.9	2	+	2	1.9
P. gl.	4	.2502	18.5	21.9	2	+	4	1.4
B. cin.		None						
Check	1		23.7					.4
Experiment 6 —10 per cent $Na_2S_2O_3$								
A. nig.	4	.2246	29.2	43.7	3	+	1	2.6
P. gl.	4	.2578	45.2	12.9	3	+	3	.5
B. cin.		None						
Check			51.9					
Experiment 7 —40 per cent $Na_2S_2O_3$								
A. nig.		None						
P. gl.	4	.5403	* 19.0	7.7	3	+	0	
B. cin.		None						
Check			* 20.6					

* One cc. of the nutrient solution was used for a titration because of the high concentration of the thiosulphate. The average of a number of determinations was taken.

and weighing the free sulphur produced as a precipitate in the flask, but all such efforts gave low results. Raciborski has reported the same difficulty in making quantitative determinations of all the sulphur introduced into the nutrient solution.

The nutrient solution for the experiments in table III was of

the composition given below with only the thiosulphate varied: KH_2PO_4 M/60, 2.2 cc.; $(NH_4)_2HPO_4$ M/6, 2.2 cc.; KNO_2 M/4, 4.7 cc.; NH_4NO_3 M/1, 1.2 cc.; $MgCl_2$ M/60, 2.8 cc.; $CaCO_3$ M/100, 0.5 cc.; $FeCl_3$ M/1000, 0.5 cc.; dextrose M/1, 6.9 cc. Of the M/1 $Na_2S_2O_3$, 10.1 cc. are used in the 5 per cent solution, 20.2 cc. in the 10 per cent solution, and 26.8 cc. of a 3/M $Na_2S_2O_3$ in the 40 per cent solution. In every case sufficient water is added to make the volume of each culture 50 cc.

In the series of increasing thiosulphate concentrations made up as 5, 10, and 40 per cent solutions, the growth of *Penicillium* was successively greater, as was the production of sulphates and H_2S, though sporulation was prevented in the solution of highest concentration. In the nutrient solutions employed, the inhibition of growth for *Botrytis* in the presence of the thiosulphate occurs between .6 and 2 per cent, for *Aspergillus* between 10 and 40 per

TABLE IV

GROWTH OF CERTAIN FUNGI ON MEDIA CONTAINING POTASSIUM THIOCYANATE AND MAGNESIUM SULPHATE. TIME INTERVAL OF CULTURES, 27 DAYS

	Experiment 8 .2 per cent KCNS				Experiment 9 KCNS replaced by $MgSO_4$			
Fungus	A. nig.	P. gl.	B. cin.	Check	A. nig	P. gl.	B. cin.	Check
No. cultures	6	5	5		5	5	4	
Dry wt. (gms.)	.3512	.1602	.4155		.9212	.4234	1.0766	
Sporulation	1	0	0		4	4	4	
H_2S	0	0	0	0	0	0	0	0

cent, while *Penicillium* grows very well on the 40 per cent solution. These statements refer to solutions in which the hydrogen-ion concentration was unknown. In the later experiments it will be shown that the toxic concentration of a salt in solution may depend to some extent on the hydrogen-ion concentration of the medium.

The nutrient solution employed was NH_4NO_3 M/1, 6.2 cc.; KH_2PO_4 M/1, 2.5 cc.; $FeCl_3$ M/1000, 0.5 cc.; sucrose M/1, 7.3 cc. in each solution, with the magnesium and sulphur supplied n experiment 8 as 1 cc. each of M/1 $MgCl_2$ and KCNS. In ex-

periment 9 the KCNS and $MgCl_2$ were omitted and the magnesium and sulphur were supplied in 1 cc. of M/1 $MgSO_4$. The potassium and chlorine content were kept practically the same by adding 1 cc. of M/1 KCl. Both growth and fructification have been greatly increased where sulphur is supplied as $MgSO_4$ instead of KCNS.

TABLE V

GROWTH OF CERTAIN FUNGI ON MEDIA CONTAINING POTASSIUM THIOCYANATE AND AMMONIUM THIOCYANATE. TIME INTERVAL OF CULTURES, 4 WEEKS

	Experiment 10 1 per cent KCNS				Experiment 11 1 per cent NH_4CNS			
Fungus	A. nig.	P. gl.	B. cin.	Check	A. nig.	P. gl.	B. cin.	Check
No. cultures	2	2	2		2	2	2	
Dry wt. (gms.)	.1313	.0159	.0288		.0445	.0125	.0250	
H_2S	1	1	3	0	1	1	3	0
Sporulation	0	0	0		0	0	0	

Nutrient solutions as employed by Kossowicz and Gröller were used and are, for experiment 10, KCNS 2/M, 2.5 cc.; KNO_3 M/1, 2.4 cc.; NH_4NO_3 M/1, 3.1 cc.; KH_2PO_4 M/6, 2.2 cc.; $MgSO_4$ M/3, 3.0 cc.; dextrose M/1, 6.9 cc. The salts in the solution for experiment 11 were NH_4CNS M/1, 6.6 cc.; KH_2PO_4 M/6, 2.2 cc.; $MgCl_2$ M/60, 7.4 cc.; $CaCO_3$ M/100, 1.0 cc.; $FeCl_3$ M/1000, 1.0 cc.; dextrose M/1, 5.6 cc. In the solutions employed, 1 per cent of both KCNS and NH_4CNS strongly inhibits the growth of the 3 organisms.

The nutrient solution for the experiments 12, 14, and 15 of tables VI and VII was the same except for the source of sulphur. The constituents of the solution were NH_4NO_3 M/1, 6.2 cc.; KH_2PO_4 M/1, 2.5 cc.; $MgCl_2$ M/1, 1.0 cc.; $FeCl_3$ M/1000, .5 cc.; dextrose M/1, 6.9 cc. The sulphur compounds were added in such quantities that the sulphur content of the solutions in the three experiments mentioned would be the same. Twenty-five cc. of M/50 $K_2S_2O_8$ were added per flask in experiment 12, 1 cc. of M/1 KSH in experiment 14, and 1 cc. of M/1 $MnSO_4$ in experiment 15. The solution to which equivalent amounts of

$KHSO_3$ were added showed no growth whatever, probably due to the high acidity as found by titration. The good growth obtained on the $KHSO_3$ in experiment 13 was in a solution where this salt was used as a source of both the potassium and sulphur with only a slight acidity produced. The solution was $KHSO_3$ M/1, 2.5 cc.; $(NH_4)_2HPO_4$ M/1, 6.2 cc.; $MgCl_2$ M/1, 1.0 cc.;

TABLE VI

GROWTH OF CERTAIN FUNGI ON MEDIA CONTAINING POTASSIUM PERSULPHATE AND POTASSIUM BISULPHITE. TIME INTERVAL OF CULTURES, 4 WEEKS

	Experiment 12 .3 per cent $K_2S_2O_8$				Experiment 13 .6 per cent $KHSO_3$			
Fungus	A. nig.	P. gl.	B. cin.	Check	A. nig.	P. gl.	B. cin.	Check
No. cultures	3		3		3	3	3	
Dry wt. (gms.)	.4183	None	.1699		.2704	.2670	.2202	
H_2S	0	0	0	0	0	0	3	0
Sporulation	1		0		4	3	0	

TABLE VII

GROWTH OF CERTAIN FUNGI ON MEDIA CONTAINING POTASSIUM HYDR O SULPHITE AND MANGANESE SULPHATE. TIME INTERVAL OF CULTURES, 4 WEEKS

	Experiment 14 .15 per cent KSH				Experiment 15 .3 per cent $MnSO_4$			
Fungus	A. nig.	P. gl.	B. cin.	Check	A. nig.	P. gl.	B. cin.	Check
No. cultures	2	2	2		2	2	2	
Dry wt. (gms.)	.3208	.1039	.2684		.4508	.3936	.4049	
H_2S	0	0	0	0	0	0	0	0
Sporulation	4	1	4		4	4	4	

$FeCl_3$ M/1000, .5 cc.; dextrose M/1, 6.9 cc. K_2S was also used as a source of sulphur with very meagre growth obtained. Under the conditions of these 4 experiments, $MnSO_4$ is the most favorable of the salts for the growth of all the organisms. The only case of complete inhibition of growth was for *Penicillium* on $K_2S_2O_8$.

B. THE USE OF THIOSULPHATE IN RELATION TO HYDROGEN-ION CONCENTRATION

The experimental work as reported in the first part of this paper failed to show any direct relation between the use of thiosulphate and the growth produced, though there were indications

TABLE VIII

AMOUNTS OF ACID OR ALKALI NECESSARY TO PRODUCE AN INITIAL P_H AS INDICATED

Cc. N/5 HCl per 50 cc. final volume	Cc. N/5 NaOH per 50 cc. final volume	Initial P_H
2.0		3.0
.3		4.2
0	0	4.5
	5.0	5.9
	15.0	7.1

TABLE IX

GROWTH AND RELATIONS OF CERTAIN FUNGI ON MEDIA CONTAINING .6 PER CENT SODIUM THIOSULPHATE. INITIAL P_H 4.2. EXPERIMENT 16

Fungus	Days	Dry wt. (gms.)	Final P_H	Cc. N/100 iodine	Per cent $Na_2S_2O_3$ decomposed	H_2S	Sulphate	Sporulation
A. nig.	5	.0719	3.8	17.4	13.8	2	—	0
	7	.1459	3.0	13.5	33.1	2	+	0
	10	.1553	2.0	11.6	42.5	2	+	0
	13	.1706	3.2	9.5	52.9	2	+	0
P. cycl.	5	.3035	3.1	12.6	37.6	1	+	0
	7	.5938	3.0	7.9	60.9	2	+	0
	10	.7374	3.2	5.3	73.7	2	+	1
	13	.6628	5.6	6.2	69.3	3	+	4
B. cin.	5	.0160	3.9	17.2	14.8	2	—	0
	7	.0416	3.9	17.1	15.3	2	—	0
	10	.1166	3.6	16.7	17.3	2	+	0
	13	.1190	3.7	13.1	35.1	2	+	0
Control			4.2	20.2		0	—	

from the tests with litmus paper and the determinations of titratable acidity that the reaction of the medium might be a factor of considerable importance. In the experiments with some of the salts other than the thiosulphate, it was also realized that rather

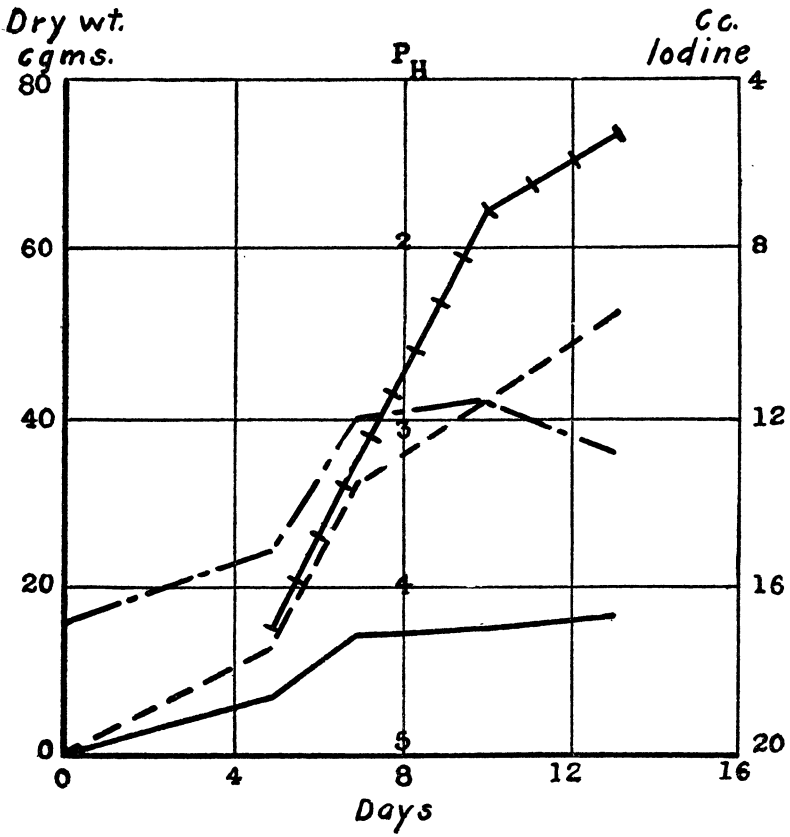

Fig. 1. *Aspergillus niger* on solution of initial P_H 4.2 containing thiosulphate.

——————— dry weight.
- - - - - - - - - - - thiosulphate decomposed expressed as cc. N/100 iodine.
— - ——— - —— hydrogen ion concentration.
—+—+—+— ratio $\frac{\text{thiosulphate decomposed}}{\text{growth}}$.

(The legend above holds for figures 1–3 and 7–15.)

acid solutions were produced in some cases, and the effect of this factor on the "toxic concentration" of the salt employed was entirely problematical. In this series of experiments the thio-

sulphate content was held constant at practically a .6 per cent solution, while the hydrogen-ion concentration was varied from an initial P_H of 3.0 to 7.1. Increases in the hydrogen-ion concentration of the original medium were obtained by the addition

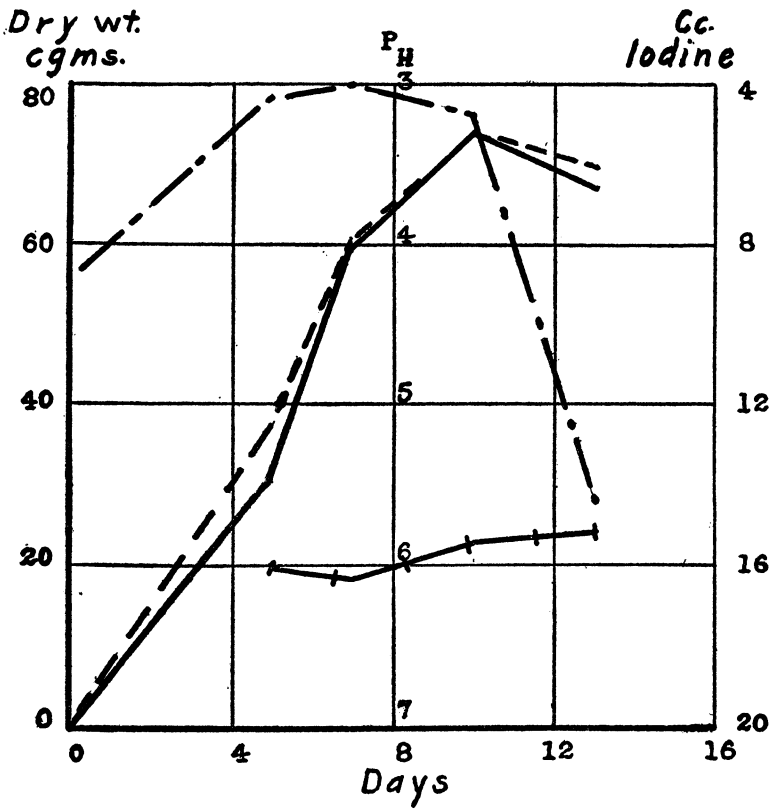

Fig. 2. *Penicillium cyclopium* on solution of initial P_H 4. 2 containing thiosulphate.

of N/5 HCl, while the decreases were obtained by the use of N/5 NaOH. If either of these compounds be added before sterilization, a caramelization and consequently a decided change in the medium occurs, so that the following procedure was adopted. The solution was mixed *en masse* for a particular series and (50—x) cc. pipetted into flasks of 120 cc. capacity which were then sterilized in an autoclave at 15 pounds pressure for 20 minutes. After cooling, x cc. of sterile acid or alkali were added under sterile conditions to each flask which was then allowed to

stand for 24 hours so that a state of equilibrium would be attained. By this procedure the final volume of the solution was 50 cc., and the concentration of the salts was comparable to that of the original solution.

The successive increases in growth, decreases in the amount of

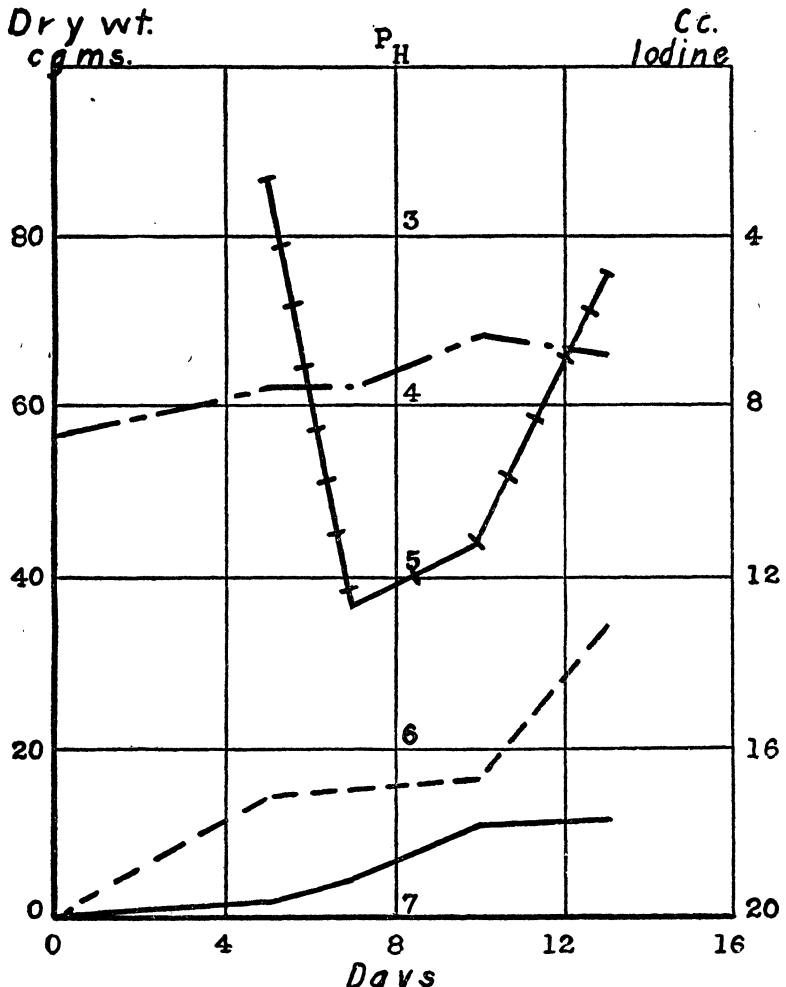

Fig. 3. *Botrytis cinerea* on solution of initial P_H 4.2 containing thiosulphate.

thiosulphate present, and changes in the reaction were determined for each series of cultures.

The composition of the medium was as follows: NH_4NO_3 M/1, 6.2 cc.; KH_2PO_4 M/1, 2.5 cc.; $MgCl_2$ M/1, 1.0 cc.; $Na_2S_2O_3$ M/1, 1.2 cc.; sucrose M/1, 7.3 cc.; $FeCl_3$ M/1000, .5 cc.; water plus acid or alkali sufficient to make 50 cc.

The addition of NaOH caused some precipitation in the medium but this was disregarded.

Experiment 16.—In following the successive changes in the cultures, quantitative determinations were made of growth, changes in P_H, and the thiosulphate content of the solution, qualitative tests for H_2S and sulphates, and observations on the sporulation of the cultures. The relations between growth, hydrogen ion, and the consumption of thiosulphate are more clearly shown in figs. 1, 2, and 3. Both *Aspergillus* and *Botrytis* show a rather meagre growth on this acid solution, while *Penicillium* grows very well, surpassing this growth in only one other solution, that of P_H 4.5. A series at P_H 3.0 was inoculated but no growth was obtained with any of the organisms.

If any direct relation exists between the decomposition of thiosulphate and the resulting growth, the ratio of thiosulphate to growth would be a constant and would appear as a straight line when plotted on coördinate paper. If the possibilities of experimental error are considered, there appears to be such a straight-line relationship in 8 of the 12 cases determined and represented in figs. 4, 5, and 6. *Penicillium* seems to exhibit the direct relationship between the decomposition of thiosulphate and growth in the series of cultures at initial P_H 4.2, while *Aspergillus* and *Botrytis* exhibit the most extreme variations. The hydrogen-ion concentration of the solution does not appear to be the factor involved in these variations, as the hydrogen-ion concentration produced by *Botrytis* was lowest during most of the experiment and yet this fungus was the most inefficient user or decomposer of thiosulphate when dry weight is considered as a criterion of efficiency. In some of the other series of experiments it may be seen that a similar low production of acidity may not similarly affect the ratio curve. A point of interest is the decided reversion of the reaction in the solution supporting the growth of *Penicillium*. This decided reversion of the reaction occurred between the tenth and thirteenth days at the period when the mycelium suddenly produced a large number of spores.

Such a reversion of the reaction at the initiation of the fruiting stage will also be noted for *Penicillium* in the series at P_H 7.1. However, no such noticeable reversions of the reaction occurred

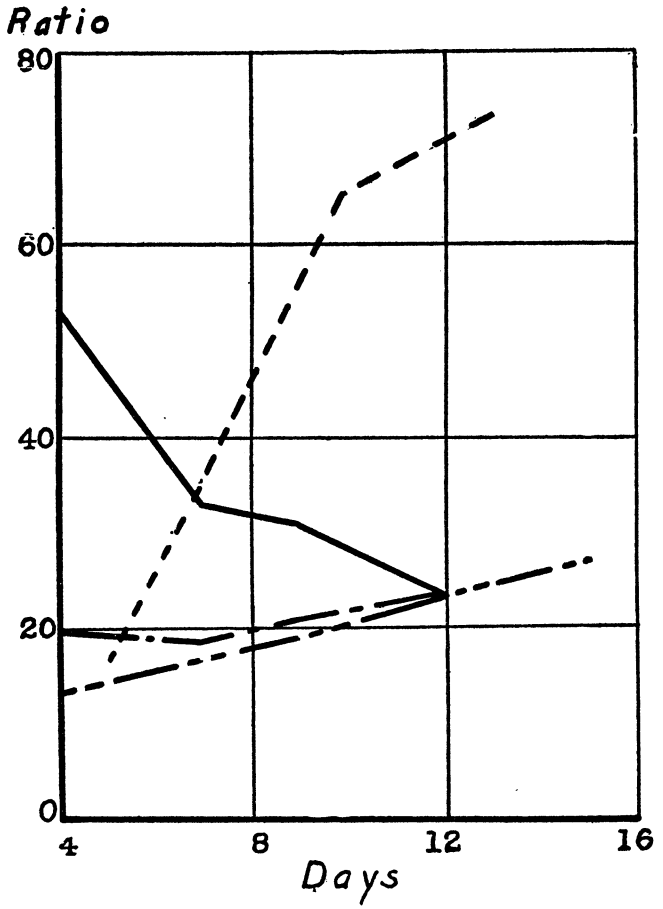

Fig. 4. *Aspergillus niger*, ratio $\frac{\text{thiosulphate decomposed}}{\text{growth}}$.
- - - - - - - - - - initial P_H 4.2.
———————— initial P_H 4.5.
—·———·—— initial P_H 5.9.
——— · · ——— · · initial P_H 7.1.
(This legend holds for figs. 4–6.)

with *Aspergillus* or *Botrytis* at any stage of growth on the solutions containing the thiosulphate.

Two products of the decomposition of the thiosulphate are

H_2S and sulphates. The control flasks, though remaining sterile throughout, had given rise to H_2S after 5 days.

TABLE X

GROWTH AND RELATIONS OF CERTAIN FUNGI ON MEDIA CONTAINING .6 PER CENT SODIUM THIOSULPHATE. INITIAL P_H 4.5. EXPERIMENT 17

Fungus	Days	Dry wt. (gms.)	Final P_H	Cc. N/100 iodine	Per cent $Na_2S_2O_3$ decomposed	H_2S	Sulphate	Sporulation
A. nig.	4	.1251	3.1	14.8	34.5	0	+	0
	7	.5258	2.1	4.4	80.5	0	+	3
	9	.6598	1.8	1.5	93.3	0	+	3
	12	.9763	1.7	.3	98.7	0	+	3
	15	.7578	1.8	.2	99.1	0	+	4
	18	.7619	1.9	0	100	0	+	4
P. cycl.	4	.0483	4.1	20.6	8.8	1	—	0
	7	.2483	3.8	17.8	21.2	1	+	0
	9	.3961	3.4	15.4	31.5	1	+	0
	12	.5143	3.6	12.4	41.3	1	+	0
	15	.7755	2.9	5.6	75.2	1	+	0
	18	.6751	2.0	5.4	76.0	1	+	1
B. cin.	4	.0643	3.8	19.5	13.7	1	+	0
	7	* .3002	3.0	10.7	52.6	3	+	0
	9	.2462	3.1	10.8	52.2	3	+	2
	12	.4148	3.0	3.8	83.1	3	+	3
	15	.7755	2.9	1.1	95.1	3	+	4
Control			4.5	22.6		† 1	0	

* 2 cultures only.

† Slight after 15 days.

Experiment 17.—The solution for experiment 17 was the original solution without the addition of acid or alkali. Notwithstanding careful technique in the preparation of the solution and the use of the best chemicals obtainable, the initial P_H of the solution varied as much as .4 P_H from time to time. More generally the reaction of the freshly prepared solution was P_H 4.9.

The increase in growth of *Aspergillus* and *Botrytis* over that obtained in the previous solution was marked, while *Penicillium*

though showing a slightly greater growth, required a third longer time to attain this. As in the former experiment, *Botrytis* decomposed a greater quantity of thiosulphate per unit weight than

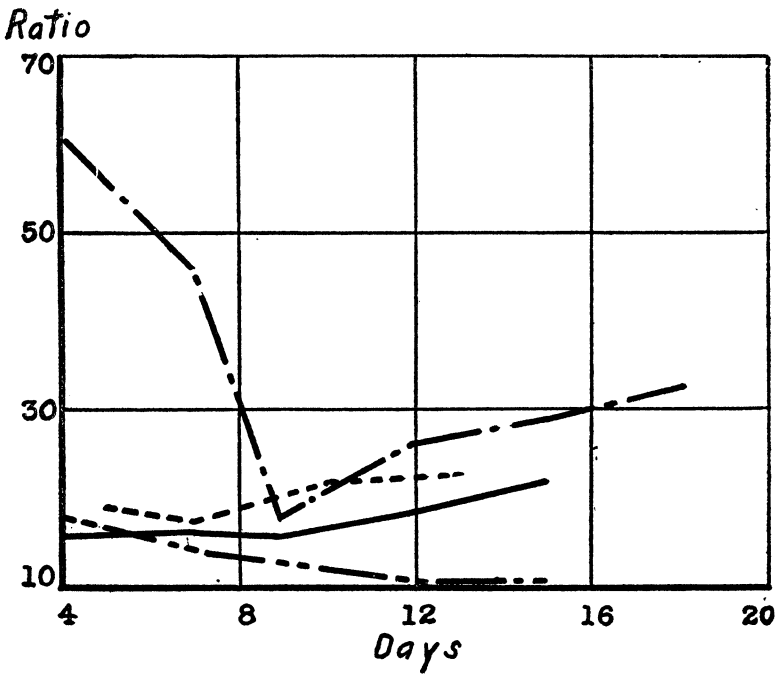

Fig. 5. *Penicillium cyclopium*, ratio $\frac{\text{thiosulphate decomposed}}{\text{growth}}$.

the other organisms and produced a greater darkening of the lead-paper, indicating a greater production of H_2S. *Aspergillus*, though producing an acidity of the solution amounting to P_H 1.7 and finally decomposing all the thiosulphate, failed to produce any H_2S. This fact is not in agreement with the idea of some earlier workers that the acidity produced in the solution might be responsible for the separation of free sulphur and the production of H_2S. The control flask produced a trace of H_2S after the fifteenth day but no sulphates were present. The results are shown more clearly in figs. 7, 8, and 9.

Experiment 18.—The chief results as presented in table XI are shown graphically in figs. 10, 11, and 12. *Aspergillus* and *Botrytis* made further increases in growth over the two previous

TABLE XI

GROWTH AND RELATIONS OF CERTAIN FUNGI ON MEDIA CONTAINING .6 PER CENT SODIUM THIOSULPHATE. INITIAL P_H 5.9. EXPERIMENT 18

Fungus	Days	Dry wt. (gms.)	Final P_H	Cc. N/100 iodine	Per cent $Na_2S_2O_3$ decomposed	H_2S	Sulphate	Sporulation
A. nig.	4	*.7956	2.1	5.5	74.3	1	+	0
	7	*1.1256	1.7	0.6	97.2	1	+	1
	9	*.9742	2.0	0.4	98.1	1	+	1
	12	*.9248	2.2	0.3	98.6	1	+	2
	15	*.8836	2.2	0.2	99.0	1	+	2
	18	*1.1650	2.3	0	100	1	+	3
P. cycl.	4	.0785	5.6	16.4	23.3	1	+	0
	7	.1855	4.9	12.9	39.7	1	+	0
	9	.4904	3.8	12.4	42.0	2	+	0
	12	.5166	3.8	7.6	64.4	2	+	0
	15	.5671	4.1	5.2	74.7	2	+	0
	18	.5771	4.2	2.5	88.3	3	+	1
B. cin.	4	*.1203	5.4	19.3	9.8	1	+	0
	7	.3526	3.5	12.4	42.0	1	+	2
	9	.6415	3.5	8.1	62.1	1	+	2
	12	1.0472	2.8	4.0	81.3	1	+	4
	15	.9991	2.6	3.4	84.1	1	+	4
	29	.8423	3.4	0	100	1	+	4
Control			5.9	21.4		0	+	

*2 cultures only.

solutions, *Aspergillus* attaining this growth very rapidly with a change in the reaction to P_H 1.7 on the seventh day. The rapid increase of growth of *Aspergillus* to the seventh day followed by a decline and a second maximum on the eighteenth day when the thiosulphate had disappeared did not occur in other cultures, so that the significance of this is not known. The unit of dry weight per unit of thiosulphate decomposed was practically the same for *Aspergillus* and *Botrytis*, with *Penicillium* exhibiting a variable ratio as shown in fig. 5. *Penicillium* was the strongest producer of H_2S though the total quantity of thiosulphate decomposed was less than for the other organisms employed.

Experiment 19.—The results as presented in table XII are shown graphically in figs. 13, 14, and 15. All the fungi made a good growth on this solution, *Aspergillus* growing rapidly, with *Botrytis* appearing so slowly that no determinations were obtain-

TABLE XII

GROWTH AND RELATIONS OF CERTAIN FUNGI ON MEDIA CONTAINING .6 PER CENT SODIUM THIOSULPHATE. INITIAL P_H 7.1. EXPERIMENT 19

Fungus	Days	Dry wt. (gms.)	Final P_H	Cc. N/100 iodine	Per cent $Na_2S_2O_3$ decomposed	H_2S	Sulphate	Sporulation
	4	.3221	3.4	16.9	23.5	0	+	1
	7	.6840	2.9	10.1	54.3	0	+	2
A. nig.	9	.9156	2.9	5.1	76.9	0	+	3
	12	.9168	2.9	2.0	90.9	1	+	4
	15	.8207	2.9	1.1	95.0	1	+	4
	4	.1864	4.4	17.6	20.3	0	+	3
	7	.3595	4.9	16.3	26.2	0	+	3
P. cycl.	9	.5026	5.1	16.3	26.2	0	+	3
	12	.6914	5.3	15.1	31.6	0	+	3
	15	.6602	5.8	15.9	28.0	0	+	4
	7	.1352	6.1	19.0	14.0	0	+	4
	9	.2687	5.4	15.7	28.9	0	+	4
B. cin.	12	.4143	5.2	12.8	42.0	0	+	4
	15	.6480	4.9	9.5	57.0	1	+	4
	18	.7861	4.7	5.6	74.6	1	+	4
Control	—	—	7.1	22.1	—	0	+	—

able at the end of 4 days. Webb ('19) has found the alkaline limit for the spores of *Botrytis* to be near the neutral point, P_H 7, and here we apparently have a case of retarded germination and growth until the change in reaction of the substratum has reached the favorable range of acidity, where a rapid increase in growth can occur. This will appear from an examination of table XIII showing the successive increases in growth of each organism in this solution. *Aspergillus* usually makes relatively large and rapid increases in growth, *Botrytis* usually attains the largest

successive increases at a later period, while *Penicillium* generally displays an intermediate condition. The ratio of the thiosulphate decomposed per unit of dry weight produced is about the

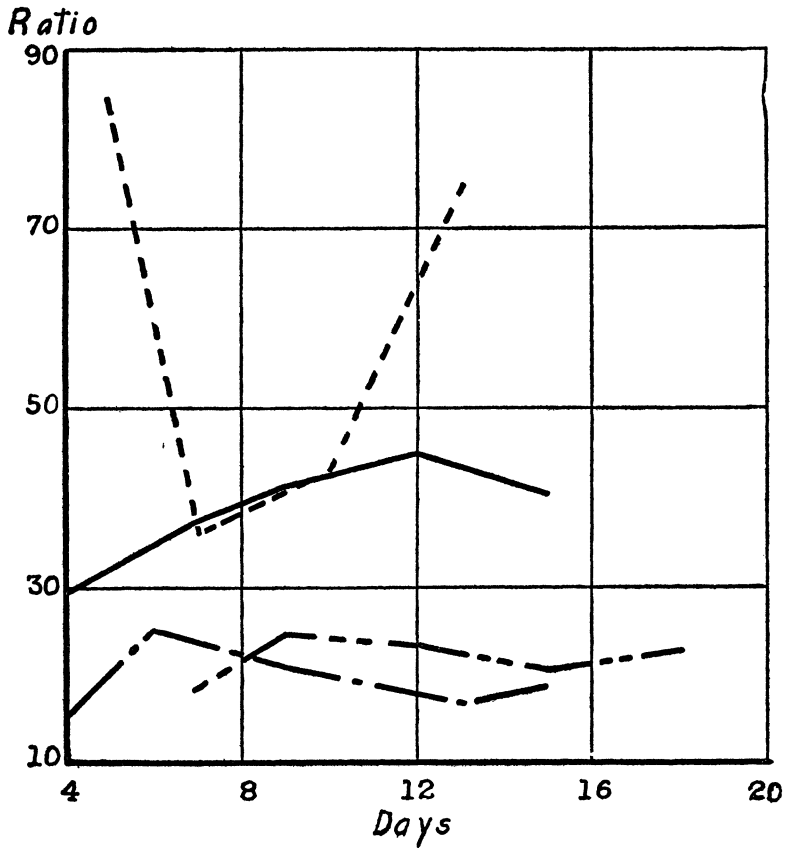

Fig. 6. *Botrytis cinerea*, ratio $\frac{\text{thiosulphate decomposed}}{\text{growth}}$.

same for *Aspergillus* and *Botrytis*, with *Penicillium* showing a more economical decomposition of the thiosulphate than the other fungi. No H_2S was produced by *Penicillium*, and there was only a slight production of this compound after the twelfth day by *Aspergillus* and after the fifteenth day by *Botrytis*. A very interesting reversion of the reaction occurred in the cultures of *Penicillium* after the first determination at 4 days, and this may have begun even earlier. No tests were made for sugar in

these cultures, though later experiments in this report will show that *Penicillium* may cause a reversion of the reaction before the sugar has disappeared.

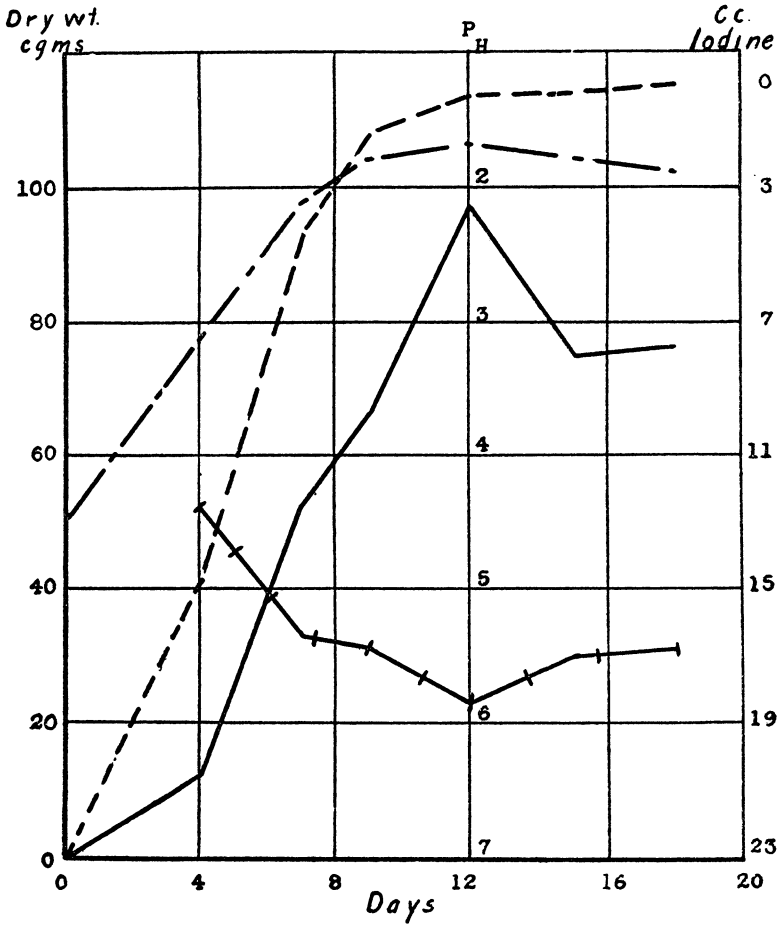

Fig. 7. *Aspergillus niger* on solution of initial P_H 4.5 containing thiosulphate.

C. GROWTH AND HYDROGEN-ION CONCENTRATION WITH $MgSO_4$ SUBSTITUTED FOR $Na_2S_2O_3$ AS A SOURCE OF SULPHUR

The basic solution in which equi-molecular weight substitutions as $Na_2S_2O_3$ were employed in the 4 preceding experiments was now used with $MgSO_4$ as the source of sulphur. To keep the

TABLE XIII

SUCCESSIVE INCREASES IN DRY WEIGHT ON THE SOLUTION WITH AN INITIAL P_H OF 7.1

Fungus	Days					
	4	7	9	12	15	18
A. nig.	.3221	.3619	.2316	.0012	—	—
P. cycl.	.1864	.1731	.1431	.1888	—	—
B. cin.	—	.1352	.1335	.1456	.2337	.1381

TABLE XIV

GROWTH AND RELATIONS OF CERTAIN FUNGI ON MEDIA CONTAINING MAGNESIUM SULPHATE. INITIAL P_H 4.1. EXPERIMENT 20

Fungus	Days	Dry wt. (gms.)	Final P_H	Sugar	Sulphates	H_2S	Sporulation
A. nig.	4	.8569	1.6	+	+	0	0
	7	1.0369	1.5	0	+	0	1
	9	.9812	1.7	0	+	0	1
	12	.9186	1.7	0	+	0	1
	15	.8723	1.9	0	+	0	1
	18	.8428	1.9	0	+	0	1
P. cycl.	4	.3232	3.7	+	+	0	1
	7	.8624	3.7	+	+	0	3
	9	.9446	4.2	+	+	0	3
	12	1.1722	4.2	Slight	+	0	3
	15	.9807	4.6	Slight	+	0	4
	18	.9344	5.0	0	+	0	4
B. cin.	4	.1915	2.9	+	+	0	3
	7	.5791	2.9	+	+	0	3
	9	.7224	2.4	+	+	0	3
	12	.8643	2.2	+	+	0	3
	15	1.0060	2.4	+	+	0	3
	18	1.0123	2.4	+	+	0	4
Control			4.1	+	+	0	

balance of sodium and chlorine ions as nearly as possible that of the former solution where $Na_2S_2O_3$ and $MgCl_2$ were used, a sufficient amount of NaCl was added to maintain the balance of sodium and change the balance of chlorine only very slightly.

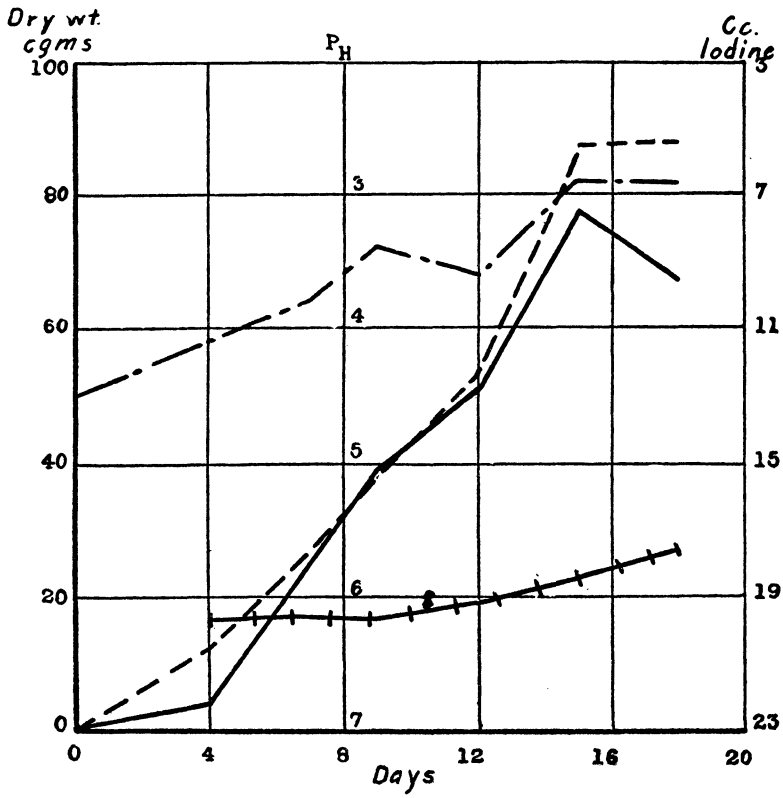

Fig. 8. *Penicillium cyclopium* on solution of initial P_H containing thiosulphate.

The modified Pfeffer's solution in which the substitutions were made is known to be a favorable solution for the growth of many fungi, hence this series of cultures was grown to determine if there had been any retarding effect of the thiosulphate in solutions of the same initial P_H, as well as the relative changes in the hydrogen-ion concentration, the production of H_2S, and the reversions in reaction in relation to the disappearance of the sugar as determined by qualitative tests with Fehling's solution. The

composition of the solution was as follows: NH_4NO_3 M/1, 6.2 cc.; NaCl M/1, 2.4 cc.; KH_2P0_4 M/1, 2.5 cc.; $MgSO_4$ M/1, 1.0 cc.; $FeCl_3$ M/1000, .5 cc.; sucrose M/1, 7.3 cc.; water, 30.1 cc.

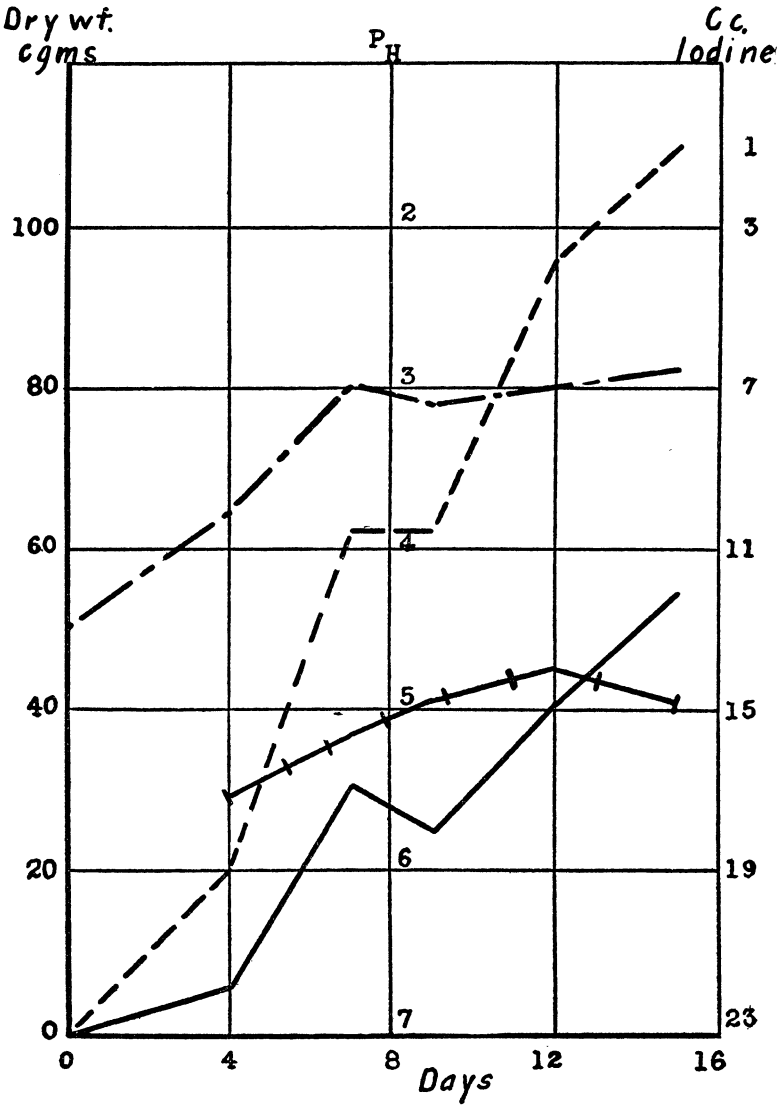

Fig. 9. *Botrytis cinerea* on solution of initial P_H 4.5 containing thiosulphate.

Experiment 20.—The solution used in this experiment, with an initial P_H 4.1, was that given above without the addition of acid or alkali. The growth of the 3 fungi is uniformly better in

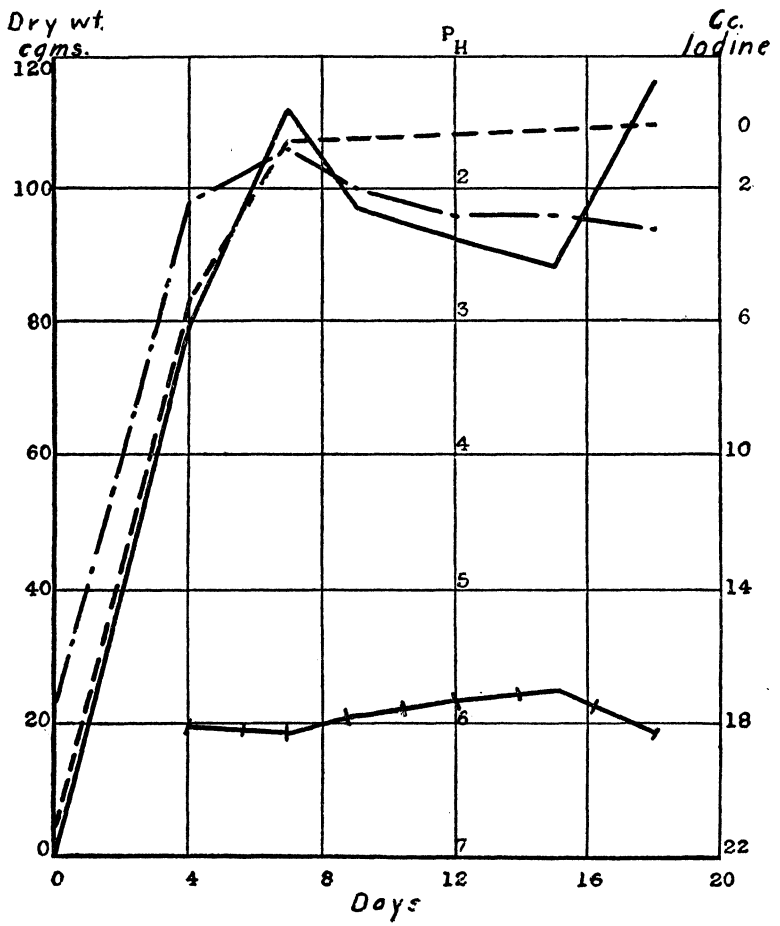

Fig. 10. *Aspergillus niger* on solution of initial P_H 5.9 containing thiosulphate.

these cultures than where the thiosulphate is used at the same P_H. This is rather markedly so for *Botrytis* which is most susceptible to the inhibiting influence of the thiosulphate. The acidity produced by *Aspergillus* and *Botrytis* is also greater than that in any of the cultures of the 4 preceding experiments.

The tests for sugar were made by adding a few cc. of Fehling's

solution to 10 cc. of the culture solution which was then warmed. If no precipitate appeared, a few drops of HCl were added to another 10-cc. portion of the culture, this brought to a boil, and Fehling's solution added as before. The latter step was not

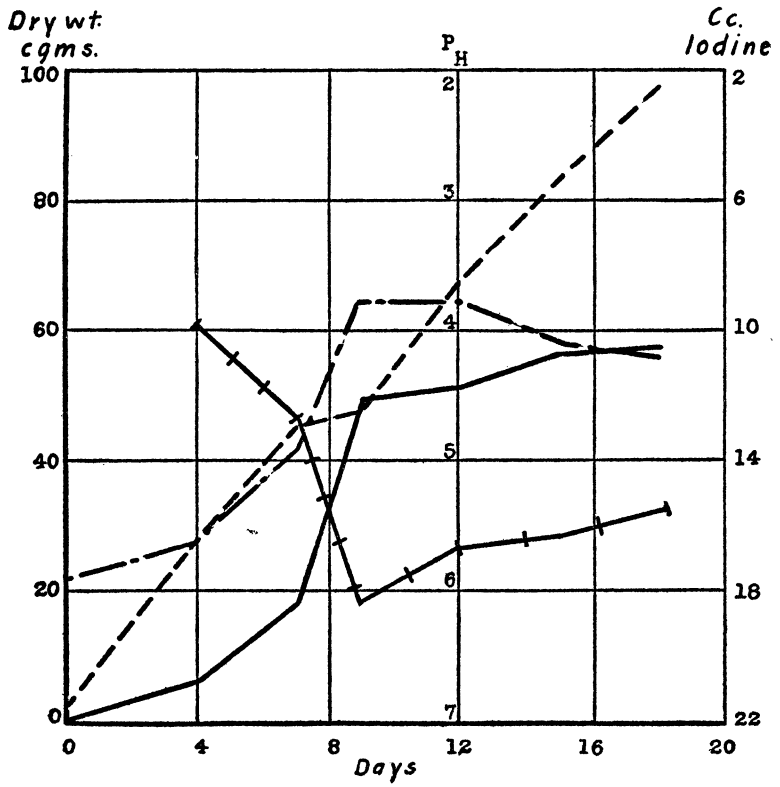

Fig. 11. *Penicillium cyclopium* on solution of initial P_H 5.9 containing thiosulphate.

necessary in any case where sugar was present, for there appeared to be some inversion even in the control flasks. A comparison of the changes in reaction caused by the different fungi can be seen from table XIV and figs. 16, 17, and 18. The peak of growth for *Aspergillus* is reached by the seventh day, with a total disappearance of the sugar and only a slight decrease in the acidity from P_H 1.5 to 1.9. The peak of growth was not reached by *Botrytis* even at the eighteenth day, neither had the sugar been

entirely used, nor was there any reversion of the reaction that might not be due to experimental error. The temperature of these experiments is not as favorable for *Botrytis* as for the other organisms, and no doubt this is one factor in its less rapid growth. The peak of growth was reached for *Penicillium* by the twelfth

TABLE XV

GROWTH AND RELATIONS OF CERTAIN FUNGI ON MEDIA CONTAINING MAGNESIUM SULPHATE. INITIAL P_H 5.5. EXPERIMENT 21

Fungus	Days	Dry wt. (gms.)	Final P_H	Sugar	Sulphates	H_2S	Sporulation
A. nig.	4	.8130	1.9	+	+	0	0
	7	1.0426	1.5	0	+	0	1
	10	.8499	2.1	0	+	0	2
	12	.7549	1.9	0	+	0	2
	15	.7409	2.9	0	+	0	3
	18	.6072	2.9	0	+	0	4
P. cycl.	4	.2466	5.3	+	+	0	0
	7	.9028	4.1	+	+	0	1
	10	1.1006	4.7	+	+	0	1
	12	1.0497	5.0	Slight	+	0	1
	15	.8440	5.0	0	+	0	1
	18	.9560	5.1	0	+	0	1
B. cin.	4	.1480	5.3	+	+	0	1
	7	.4881	4.3	+	+	0	3
	10	.7611	3.6	+	+	0	3
	12	.8719	2.9	+	+	0	3
	15	.8645	2.9	+	+	0	3
	18	.9728	3.1	+	+	0	3
Control			5.5	+	+	0	

day, with sugar present in small amounts the fifteenth day, though a reversion of the reaction began after the seventh day and proceeded from P_H 3.7 to 5.0, when the sugar had disappeared from the solution.

Experiment 21.—To produce a solution with an initial P_H of 5.5, 5 cc. of sterile NaOH were added to each flask, hence this quantity of water was deducted from that added to each flask in

experiment 20. The different relations of the fungi to the changes produced can be seen in table xv and figs. 19, 20, and 21. In comparing these results with those in experiment 18 where the same amount of sodium hydroxide was added to the thio-

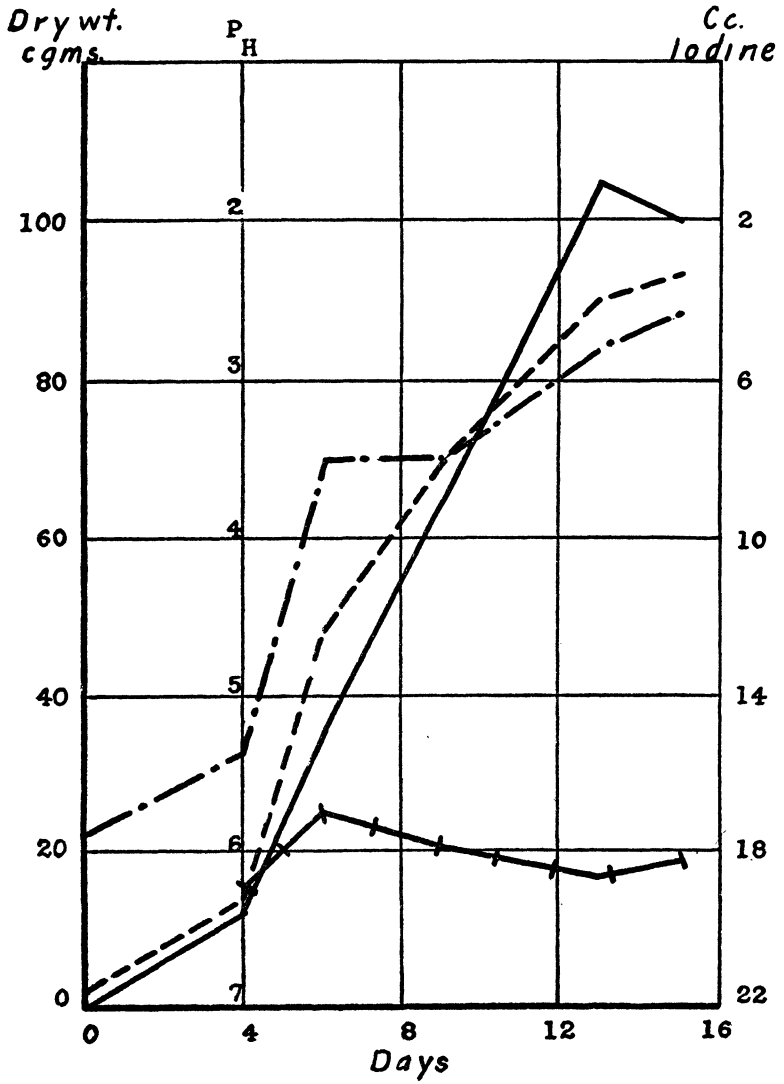

Fig. 12. *Botrytis cinerea* on solution of initial P_H 5.9 containing thiosulphate.

sulphate cultures, however with a slightly different P_H, it can be seen that the growth of *Aspergillus* is less, that of *Botrytis* about the same, and that of *Penicillium* practically double. Both *Aspergillus* and *Penicillium* exhibit a reversion of the reaction,

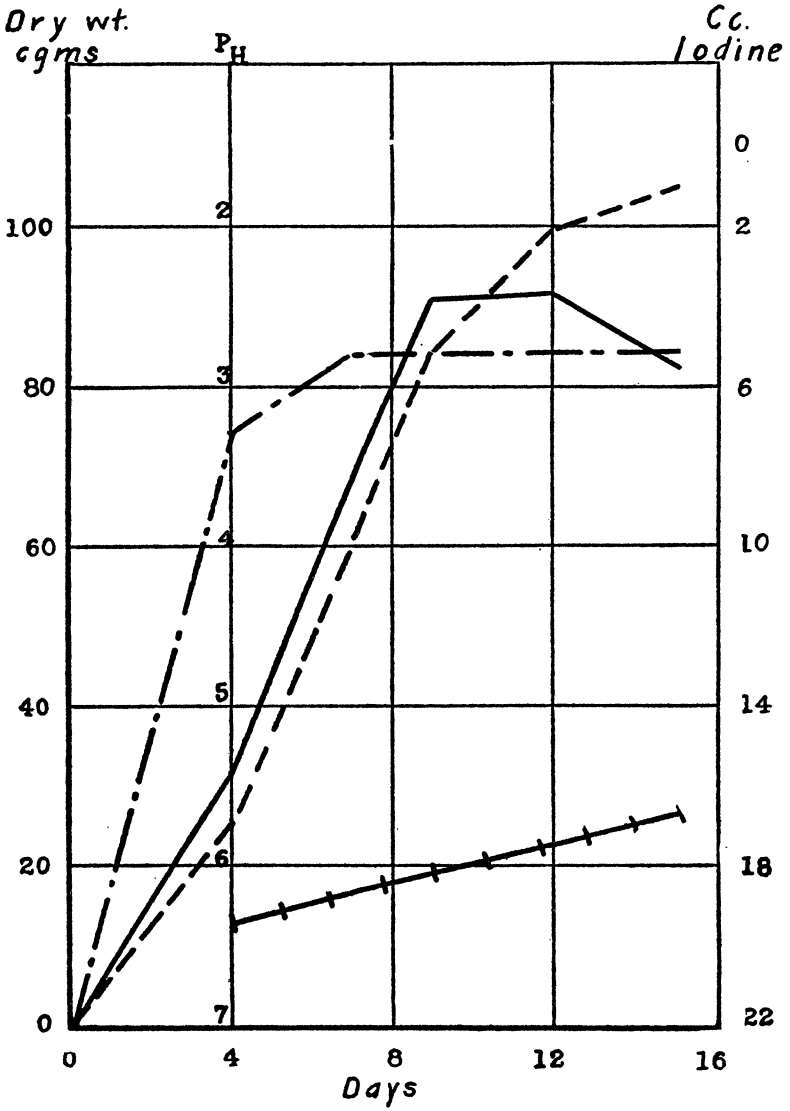

Fig. 13. *Aspergillus niger* on solution of initial P_H 7.1 containing thiosulphate.

the reversion becoming apparent in cultures supporting *Aspergillus* after the seventh day, when sugar was no longer present; while the reversion with *Penicillium* occurred at the same period, though sugar was present until after the twelfth day. The change in reaction for *Aspergillus* was from P_H 1.5 to 2.0 and for *Penicillium* from P_H 4.1 to 5.1.

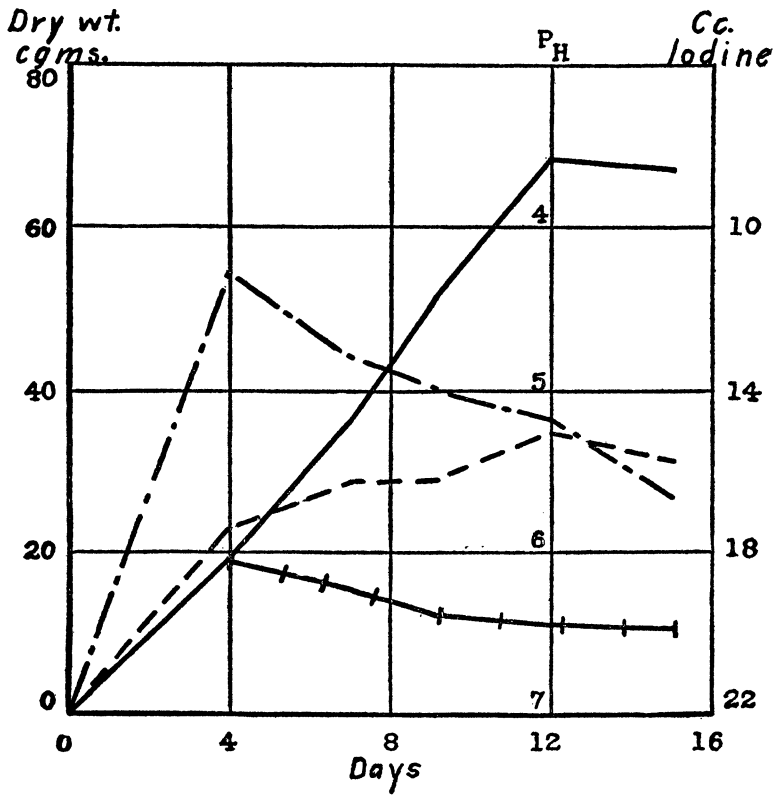

Fig. 14. *Penicillium cyclopium* on solution of initial P_H 7.1 containing thiosulphate.

No H_2S was produced from any culture where magnesium sulphate served as a source of sulphur.

Discussion

In all the cultures with $Na_2S_2O_3$ as a source of sulphur, sulphates appear as the chief end product of the action of the fungus

on this compound, H_2S is generally produced, and extracellular sulphur, the tetrathionate, and also globules of sulphur in the hyphae sometimes occur.

Nathansohn ('02), Tanner ('17), Beijerinck ('00, '04), Neuberg

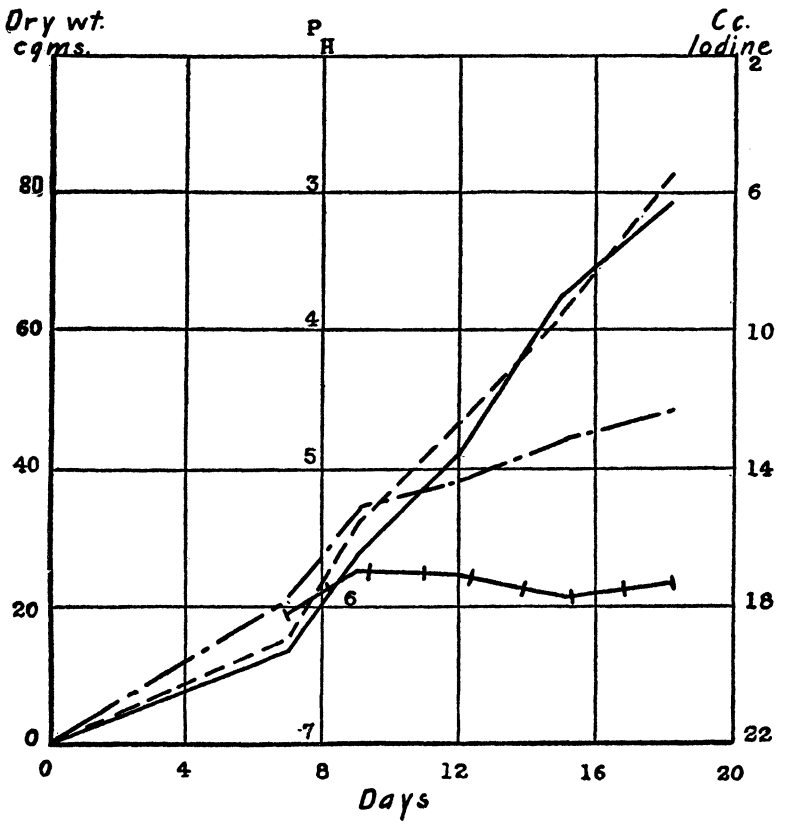

Fig. 15. *Botrytis cinerea* on solution of initial P_H 7.1 containing thiosulphate.

and Welde ('15), and Raciborski ('05), working with bacteria and higher fungi, have presented equations representing the possible course of the changes involved, the variations in the equations depending upon the chief end product. The 3 fungi used have produced all of the above-named compounds in one or the other of the solutions employed, hence it has seemed inadvisable to give a specific equation representing the probable nature of the metabolic changes.

The production of H_2S has seemed unrelated to any of the factors determined in these experiments, such as hydrogen-ion

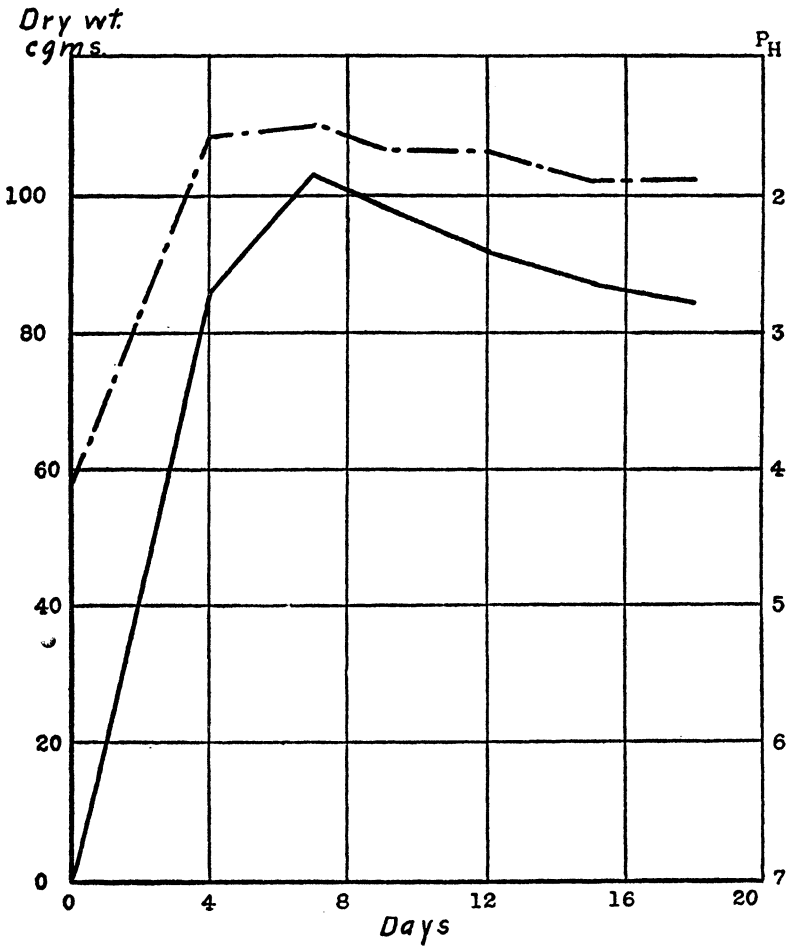

Fig. 16. *Aspergillus niger* on solution of initial P_H 4.1 containing magnesium sulphate.

——————— dry weight.

———·——·— hydrogen-ion concentration.

(The legend above holds for figs. 16–21.)

concentration, concentration of the salt, relative decomposition of the salt, or relative degree of growth. This compound is generally produced, but the rather similar conditions under which it sometimes fails to occur make it difficult to relate clearly its

production to the known factors. For example, *Aspergillus* has produced H_2S from every solution containing thiosulphate except the one with initial P_H 4.5, lowest P_H 1.7, where all the

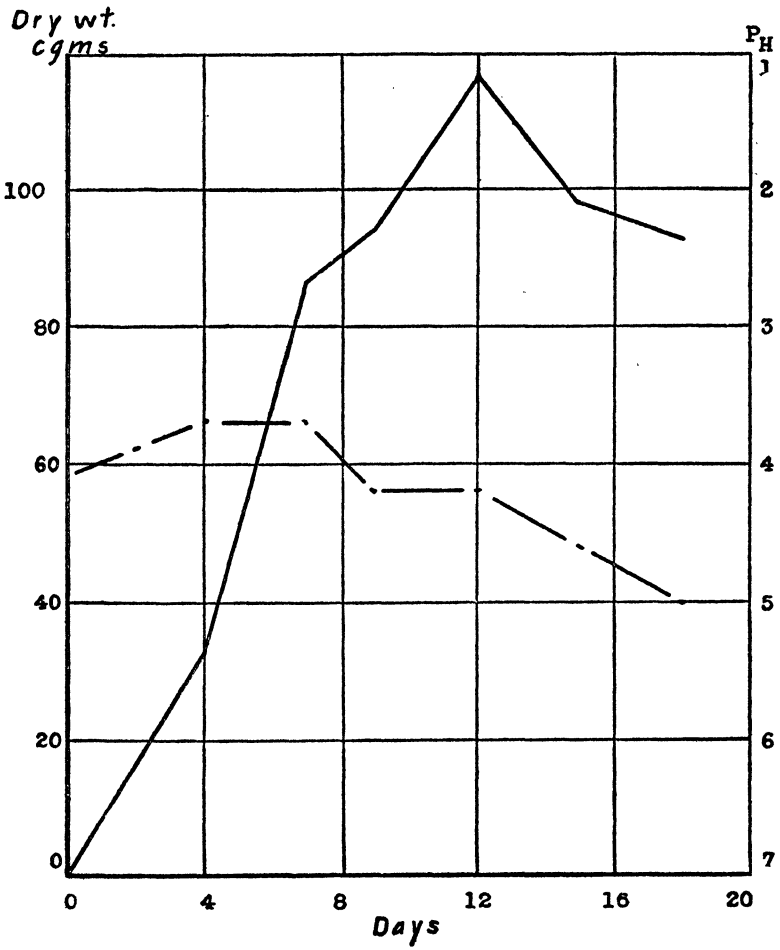

Fig. 17. *Penicillium cyclopium* on solution of initial P_H 4.1 containing magnesium sulphate.

thiosulphate was decomposed and sulphates were clearly apparent. *Penicillium* has behaved similarly except on the solution with the initial P_H 7.1, lowest P_H 4.4, where the growth was good, yet with little of the thiosulphate decomposed. The initial acidity alone might be conceived as the limiting factor, for the

actual acidity during much of the growing period was practically the same in the two cases cited as for other experiments.

A separation of free sulphur has occurred, more particularly at the higher concentrations. That the deposit is sulphur, or

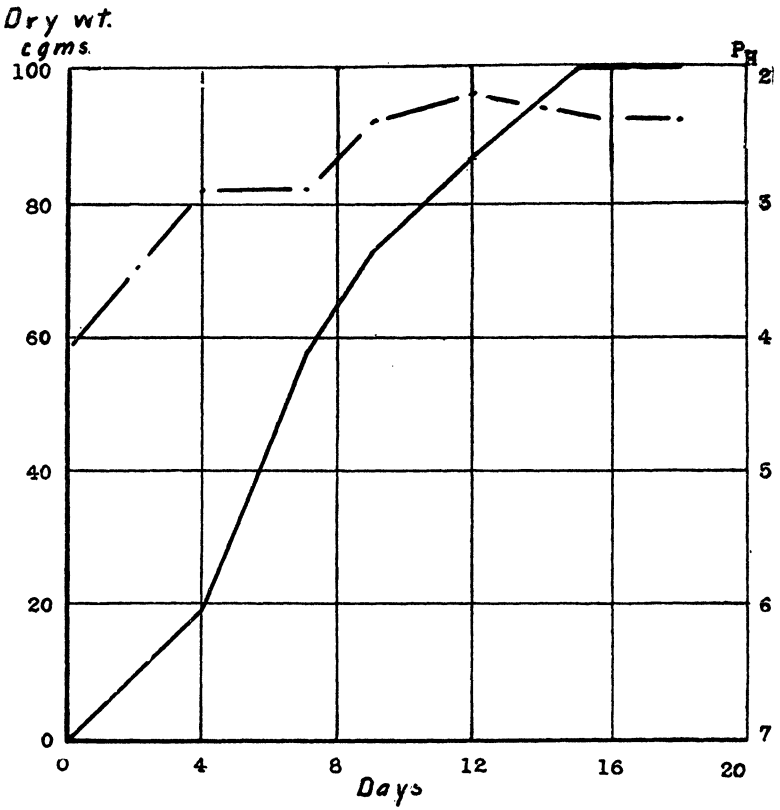

Fig. 18. *Botrytis cinerea* on solution of initial P_H 4.1 containing magnesium sulphate.

largely so, is shown by the easy solubility of most of the deposit in CS_2 and by the distinct odor of sulphur obtained on drying and heating the deposit. Globules like those in milk of sulphur, a well as many rhombic and monoclinic crystals, were found in the cultures with the higher concentrations of the thiosulphate. Nathansohn ascribed the separation of sulphur in his cultures to the production of a tetrathionate, while Raciborski could find no tetrathionate but observed the separation of sulphur. In the

solution employed in experiment 3 in which *Aspergillus* evidently caused the production of a tetrathionate, there was no apparent separation of sulphur, while in several other cultures where the separation occurred no tetrathionate could be detected.

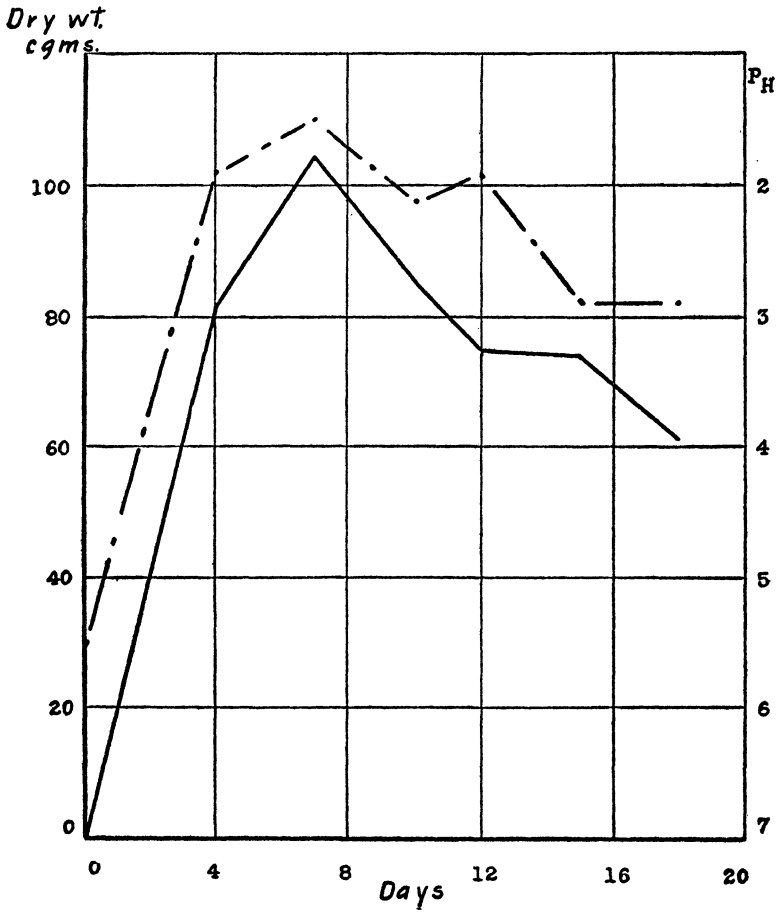

Fig. 19. *Aspergillus niger* on solution of initial P_H 5.5 containing magnesium sulphate.

The tips of many hyphae contained globules of varying sizes after a growth period of 21 days in the 5 and 10 per cent solutions of thiosulphate. A few days after the end of this period, some of the globules were found crystallizing in the hyphae in the shape of double pyramids as described by Molisch ('13) and Miyoshi

('97). These globules and crystals give the reactions and have the appearance of sulphur, and they show that these fungi can, under certain conditions, accumulate sulphur in the mycelium as

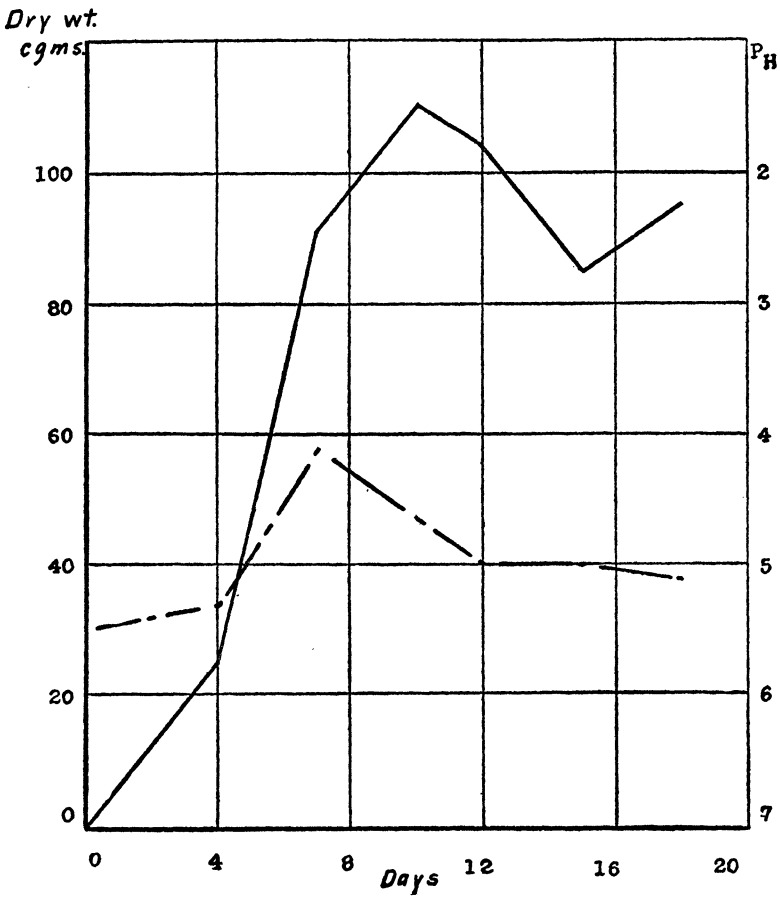

Fig. 20. *Penicillium cyclopium* on solution of initial P_H 5.5 containing magnesium sulphate.

in the case of the filamentous sulphur bacteria. The solubility of the globules in carbon bisulphide, ether, and chloroform, and their insolubility in hydrochloric acid, absolute alcohol, hot alkali (KOH), glacial acetic acid, nitric acid, benzol, and benzol after treatment with absolute alcohol, indicate that they are sulphur.

In experiments 16–19 inclusive, in which the thiosulphate was used as a source of sulphur, the ratio of thiosulphate decomposed to growth is not a constant in all cases, but the constant relation does appear in 8 of 12 sets of cultures as shown in figs. 4, 5, and 6. The hydrogen-ion concentration of the medium does not appear to be a limiting factor in the efficiency of the use of the thiosulphate.

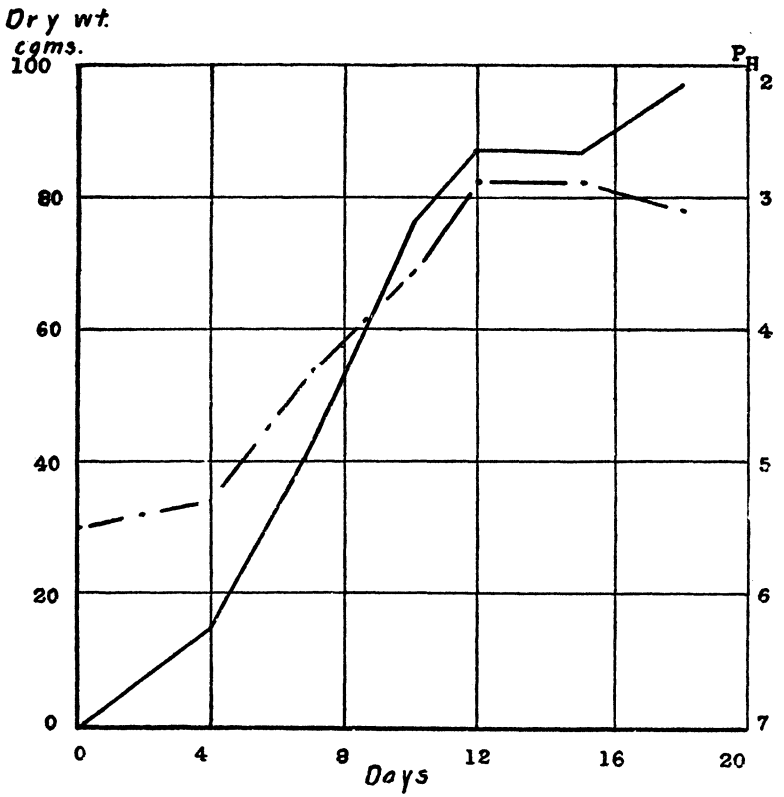

Fig. 21. *Botrytis cinerea* on solution of initial P_H 5.5 containing magnesium sulphate.

Reversions of the reaction from the more acid condition towards neutrality were observed with both *Aspergillus* and *Penicillium*. From the results of experiments 20 and 21 it is seen that *Aspergillus* caused a rapid decomposition of the sugar, that the total disappearance of the sugar is accompanied by the production

of high acidity, and that a reversion of the reaction immediately follows. *Penicillium cyclopium* caused the reversion to take place before the disappearance of the sugar, suggesting the simultaneous production of hydrogen and hydroxyl ions with the hydroxyl ions produced in excess. Chambers ('20) has studied some interesting relations of the effect of the concentration of the sugar upon the reversion of the reaction by *Bacillus coli* and *Bacillus aerogenes*. Ayers and Rupp ('18) have explained the simultaneous production of acid and alkali by *B. aerogenes* in an inorganic medium as due to the production of organic acids from the sugar with the subsequent formation of alkaline carbonates or bicarbonates from the organic acids.

The results of the determinations of the hydrogen-ion concentration of the medium at relatively short intervals make it apparent that the method of determining the initial and final hydrogen-ion concentrations of fungous cultures may not give an indication of the changes which have proceeded in the reaction.

Sporulation was retarded or largely inhibited in the more acid solutions. *Aspergillus niger* produced the greatest acidity and sporulated at a higher acidity (e. g., P_H 1.7–2.1) than either *Penicillium* or *Botrytis*. The heavy sporulation of *Penicillium cyclopium* in the solution with an initial P_H 4.2 occurred during the rapid reversion of the reaction when the acidity of the solution was P_H 3.0 or above.

Conclusions

1. $MgSO_4$, $Na_2S_2O_3$, $MnSO_4$, KSH, $KHSO_3$, $K_2S_2O_8$, KCNS, and NH_4CNS, in general, have served as favorable sources of sulphur, in the order named, for *Aspergillus niger*, *Penicillium glaucum*, and *Botrytis cinerea*. Meagre growth was obtained with K_2S. Inhibition of growth occurred for *Penicillium* on $K_2S_2O_8$ though this compound was better for *Aspergillus*, in the concentration employed, than KSH or $KHSO_3$.

2. H_2S has been produced except where $MnSO_4$, $MgSO_4$, and $K_2S_2O_8$ were used. The production of this compound seems unrelated directly to hydrogen-ion concentration, concentration of the salt, or relative degree of growth.

3. In the culture solution, sulphates appear as the chief end product of the action of the above-named fungi on $Na_2S_2O_3$,

H_2S is generally produced, molecular sulphur in visible quantity not infrequently appears, the tetrathionate has been identified in certain cases, and in the hyphae globules of sulphur sometimes occur.

4. The ratio of thiosulphate decomposition to growth is not a constant in all cases for *Aspergillus niger*, *Penicillium cyclopium*, and *Botrytis cinerea*, though in the 12 series of cultures here reported upon such a constant relation does appear with one or more of the fungi in 8 of the series. The usual growth range of hydrogen-ion concentration does not appear to be a limiting factor in the efficiency of the thiosulphate as a source of sulphur for these fungi.

5. In a modified Pfeffer's solution the disappearance of the sugar, within the limits determined, marks the point of the reversion of reaction for *Aspergillus niger*. *Penicillium cyclopium*, on the other hand, may cause a reversion of the reaction with sugar present in the solution.

6. Since it has been established that reversion of the reaction may occur, it is clear that the true course of the changes which have occurred may not be obtained merely by a determination of the initial and final hydrogen-ion concentrations of the fungous cultures.

The writer wishes to express his appreciation of the invaluable suggestions and criticisms of Dr. B. M. Duggar in the later investigations which are the subject of much of this paper. Thanks are extended to Dr. J. B. Overton, of the Department of Botany of the University of Wisconsin, for helpful direction and advice in the early progress of this work. Thanks are also due Dr. George T. Moore for the privileges and facilities of the Missouri Botanical Garden.

Graduate Laboratory, Missouri Botanical Garden.

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